Duplication at chromosome 2q31.1-q31.2 in a family presenting syndactyly and nystagmus.

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ABSTRACT

HOXD genes encode transcription factors involved in the antero-posterior patterning of the limb bud and in the specification of fingers. During the embryo development, *HOXD* genes are expressed following a spatio-temporal colinearity which involves at least 3 regions, centrometric and telomeric to this cluster. Here, we describe a father and a daughter presenting a 3-4 hand bilateral syndactyly associated with a nystagmus. Array-CGH showed a 3.8 Mb duplication at 2q31.1-q31.2 comprising 27 genes including the entire *HOXD* cluster. We performed expression studies in lymphoblasts by RT-PCR and observed a *HOXD13* and *HOXD10* overexpression whereas the *HOXD12* expression was decreased. *HOXD13* and *HOXD10* overexpression associated with a misregulation of at least *HOXD12* may therefore induce the syndactyly. Deletions of the *HOXD* cluster and its regulatory sequences induce hand malformations and particularly finger anomalies. Recently, smaller duplications of the same region have been reported in association with a mesomelic dysplasia, type Kantaputra. We discuss the variable phenotypes associated with such 2q duplications.

Key Words:

Syndactyly, HOXD cluster, 2q31.1q31.2 duplication

INTRODUCTION

HOXD genes encode a family of highly conserved transcription factors involved in the antero-posterior patterning of the limb bud and in the specification of fingers [1,2]. During embryo development, *HOXD* genes are expressed following a spatio-temporal colinearity involving at least 3 regulatory regions, centrometric (ELCR) and telomeric (POST and Global Central Region (GCR)-Prox) to the cluster [3]. Moreover, these genes are expressed through two waves [4] during the limb budding and control the patterning of the stylopod and the zeugopod [5]. The width and the efficiency of the genes' expression depend on their rank in the cluster. Each gene presents a precise pattern of expression. *HOXD13*, the most 5' end located gene, is highly expressed throughout the presumptive digits whereas *HOXD10*, *HOXD11* and *HOXD12* are restricted to presumptive digit 2 to 5 and are underexpressed. In man, deletions of this cluster induce hand malformations and particularly finger anomalies [5]. Deletions of the whole cluster can cause severe defects while deletions removing only *HOXD9-HOXD13* are responsible for a milder phenotype including fifth finger clinodactyly, variable cutaneous syndactyly of toes, hypoplastic middle phalanges of the feet and synpolydactyly [6] [5]. Deletions removing GCR are deleterious too but induce minor anomalies [7-9]. ELCR has not been localised so far. Its role is so critical that deletions would be lethal and thus there is no animal model.

Animal models carrying internal duplications of part of the *HOXD* cluster and limb anomalies exist [3,4]. Indeed, mice with targeted disruptions of *Hoxd11* and *Hoxa11* genes showed marked zeugopod malformation [10]. A disconnection of *5' Hoxd* genes from the regulator could result in a downregulation of *5' Hoxd* genes in the distal limb (autopod) and an upregulation in the proximal limb, and it has been suggested that the

2q duplication could have the same effect, therefore explaining the mesomelic dysplasia recently reported [11,4] .Indeed, it has been recently reported that a 1Mb microduplication of *HOXD* gene cluster at 2q31.1 is associated with a dominant mesomelic dysplasia, Kantaputra type. The condition is mainly affecting the upper limbs, and is very variable among affected patients within the same family. This phenotype, linked with a small 2q duplication that contain the entire *HOXD* cluster, is far more severe than the one we report here, in which the duplicaton is larger and involving several other genes.

Indeed, we report on a father and his daughter referred to the genetic clinic for the association of bilateral 3-4 finger cutaneous syndactyly and nystagmus carrying a 2q31.1q31.2 duplication involving 27 genes, among which the whole *HOXD* cluster, identified by array-CGH. We characterise this chromosomal anomaly and discuss the genotype-phenotype correlations.

PATIENTS AND METHODS

The probands are a father and his daughter. The father presents an association of bilateral 3-4 hand cutaneous syndactyly and a pendular-resilient nystagmus which increases in up and right gaze and decreases in down gaze. Ophthalmologic examination and functional tests were normal (slit-lamp, fundoscopy, binocular visual field and electroretinogram), as well as a full neurological examination. Skeletal survey including hand x-rays was normal (Figure 1A). His six-year-old daughter is affected with the same hand malformations (Figure 1B). She was born after an uneventful pregnancy. Motor milestones were achieved normally and her psychomotor development is in correlation with her age. Her growth parameters are advanced (123 cm, +2.5 SD for the height; 29 kg, + 3 SD for the weight and 51 cm, -0.5 SD for the head circumference). No dysmorphic features were identified. She presents a slow pendular nystagmus which

causes amblyopy (confirmed by visual evoked potentials). A full neurological examination was normal as well as a skeletal survey including hand x-rays (Figure 1C).

Genomic DNA was extracted from peripheral blood lymphocytes for both patients and the unaffected mother. Detection of gene copy number was performed by array-Comparative Genomic Hybridization (CGH) (Agilent™, Agilent Technologies, Santa Clara, CA) using 44 000 oligo probes approximately spaced at 40-100 kb intervals across the genome (Human Genome CGH microarray 44B kit, Agilent™). Male and female genomic DNAs (Promega™) were used as reference in hybridisations which were analysed with the CGH-analytics software by applying a Z-score segmentation algorithm to identify chromosome aberrations.

Q-PCR was performed on genomic DNA extracted from the 3 members of the family. TaqMan analyses were performed in Fast Gene Quantification in 96-Well Pates. The final volume was 20µl and contained Genotyping master Mix (15µl), 4µl of DNA and 1µl of specific genes' primers and probes. Two exons per gene were studied: *HOXD13* (exon 1 and 2), *HOXD12* (exon 1 and 2), *HOXD10* (exon 1 and 2), *CHN1* (exon 1 and 13), *CHRNA1* (exon 2 and 10). All reactions were performed in triplicate. Thermal cycling conditions were as follows: denaturation at 95°C for 10 min and 40 cycles of 95°C for 15 s; 60°C for 60 s; 72°C for 60 s. Analyses were carried out on a 7900HT Sequence Detection System and interpreted with the comparative Ct methods. *RNASEP* was used as the reference gene control.

Total RNA was extracted from lymphoblasts cell cultures, using the RNeasy Mini Kit (Qiagen GmgH, Hilde, Germany) following the manufacturer's instructions. A total of 2µg of RNA was retrotranscripted into cDNA, with the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City), for 1 hr at 37°C. TaqMan analyses were

performed in Fast Gene Quantification in 96-Well Pates. The final volume was 20µl and contained Gene Expression master Mix (10µl), 2µl of cDNA and 1µl of specific Gene Expression Assays for human *HOXD13*, *HOXD12*, *HOXD10*, *CHN1*, *CHRNA1* and *RPL13A* (primers and probes sequences Applied Biostystems trademarks), following manufacturers' instructions. All reactions were performed in quadruplicate. Thermal cycling conditions were as follows: denaturation at 95°C for 10 min and 40 cycles of 95°C for 15 s; 60°C for 60 s; 72°C for 60 s. Analyses were carried out on a 7900HT Sequence Detection System and interpreted with the comparative Ct methods. *RPL13A* was used as the reference gene control.

Chromosomal analyses of peripheral blood lymphocytes according to routine procedures using GTG-banding (550 bands) and FISH analyses using bacterial artificial chromosome (BAC) clone RP11-483E17 localized at 2q31.1 (chr2:175,041,497-175,231,429) and clone RP11-250N10 localized at 2q31.2 (chr2:178,079,879-178,252,293) (hg18, NCBI Build 36) were performed in both patients.

RESULTS

We report on a father and his daughter presenting a congenital nystagmus and a 3-4 hand bilateral syndactyly. Array-CGH identified a 3.8 Mb wide 2q31.1q31.2 duplication which comprises 27 genes and involves the whole *HOXD* cluster, *CHN1* and *CHRNA1* and also a large portion of local chromosome environment (Figure 2).

We studied the level of cDNA in lymphoblasts to evaluate the impact of the duplication on the involved genes' expression. Q-PCR analysis confirmed the duplication of *HOXD13*, *HOXD12*, *HOXD10*, *CHN1* and *CHRNA1*. The analysed exons of each tested gene were double dosed in the duplicated patients comparatively to the unaffected mother (Figure 3). *HOXD13* and *HOXD10* were overexpressed in the father (3.7 and 2.9

fold respectively) and his daughter (3.0 and 6.2 fold respectively). The expression of *HOXD12* was diminished in the daughter (5 fold) but in the father no difference was shown (data not shown).

Chromosome analyses (550 bands) were normal and FISH analyses revealed direct 2q31.1q32.2 duplication in both patients (Figure 4).

DISCUSSION

We report on a father and his daughter presenting a large 2q31.1 duplication involving the *HOXD* cluster, but also many other genes, and a very mild phenotype, namely a cutaneous syndactyly between two fingers and a nystagmus. Recently, two reports on a dominant mesomelic dysplasia type Kantaputra have been described in association with a 2q31.1 duplication involving the *HOXD* locus and other genes (*MTX2, EVX2, KIAA1715*) out of which some are also known to have important roles during digit development [14,11]. The patients presented severe shortening of the middle segments of the arm, relative shortening of the tibia and fibula and no ophthalmological associated anomaly. Since our cases had a normal full skeletal survey, their phenotype is very different and is restricted to a bilateral cutaneous syndactyly between the 3rd and 4th fingers. The 2q31.1 duplication in our cases was larger than that reported by the previous authors [14,11]. We do not know whether our cases' phenotype is linked with increased gene expression or dysregulation at the *HOXD* locus. *HOXD13* overexpression might explain the cutaneous syndactyly, although further expression studies in cells from the developing autopod rather than in lymphoblasts would be needed to ascertain this. It has been suggested that the ELCR could be needed to implement colinear expression of the *HOXD* cluster [3]. The duplication could disconnect the cluster from the ELCR and therefore explain the limb phenotype, although the

recent report from Kantaputra et al. did not identify the same 2q duplication in other affected individuals and suggested that a balanced structural chromosomal rearrangement affecting *HOXD* locus regulation could also explain the phenotype [11].

A modification of the chromosomal environment due to the duplication could possibly be involved in the genesis of the finger phenotype. Such mechanisms have already been described by Dlugaszewska et al. and correspond to translocations and inversions with breakpoints near the *HOXD* cluster [13]. Patient 2 (as designated in the original article) carried a t(2;10)(q31.1;q23.33) translocation with a proximal breakpoint around 1050 kb downstream to *HOXD13*. He harboured ulnar hypoplasia and absence of fingers 3 to 5 and hypoplastic fingers 1 and 2. In this case, a first wave impairment could be suspected because of the zeugopod involvement and thus ELCR misregulation might be involved in the phenotype. Regarding our patients, karyotype and FISH analyses allowed to confirm that the duplication was tandem rather than being translocated to another chromosome.

It is known that *HOXD* products need to be adequately balanced for a normal digit pattern. Thus, we studied the expression of *HOXD13*, *HOXD12* and *HOXD10* in 2 affected patients carrying a 2q31.1q31.2 duplication. We showed that, in lymphobasts, the duplication was responsible for a complex modification of *HOXD* genes' expression and we hypothesised that this may alter the limb bud development and cause the phenotype.

Indeed, the overexpression of the most 5' located *HOXD* gene can, by itself, generate finger anomalies. It has been demonstrated that in presence of *GLI3, HOXD10* up regulation induces polydactyly, whereas up regulation of *HOXD13* and *HOXD12* leads to oligodactyly [12]. Moreover, the altered expression of *HOXD* genes probably modifies

193 their pattern of expression and impairs the digit shaping as described in an animal
194 model presenting an internal duplication of the complex [3,4]. The overexpression of
195 *HOXD10* and *HOXD13* modifies the ratio between 5' *HOXD* genes and *GLI3* products,
196 probably mimicking a lack of *GLI3* products that corresponds to Greig syndrome in
197 which cutaneous syndactyly occurs.
198 For the genes presumptively involved in the nystagmus, *CHN1* was over expressed in
199 the duplicated patients (2.1 fold in the father and 1.6 fold in the daughter) as well as
200 *CHRNA1* (1.3 fold in the father and 3.0 fold in the daughter) (data not shown).
201 Ocular motility depends on the precise innervation of ocular motor muscles. Abnormal
202 innervation can give rise to nystagmus. The *CHN1* gene encodes two Rac-specific
203 guanosine triphosphatase (GTPase)-activating a-chimaerin isoforms. Miyake et al.
204 recently identified missense mutations in *CHN1* which induce a gain of function of
205 alpha2-chimerin and cause aberrant innervation of oculomotor muscles in animal
206 models [15]. Thus, overexpression of this gene in our patients might cause hyperactivation
207 of a-chimaerin and impair normal ocular motor innervation, although Duane syndrome
208 is distinct from nystagmus and we can not prove this. The effect of this overexpression
209 could possibly be modulated by the overexpression of *CHRNA1*. No other candidate
210 gene seemed to be potentially associated with eye anomalies in the duplicated region.
211 Another explanation could be the dysregulation of a gene, distant from the duplication,
212 which we have not identified yet.
213 We show that duplication of the *HOXD* cluster disturbs, at least, *HOXD10*, *HOXD12*
214 and *HOXD13* expression. This misregulation possibly gives rise to syndactyly through a
215 direct effect of excessive *HOXD* genes' products, or because of ratio disequilibrium
216 between 5' *HOXD* and *GLI3* products. In addition, the modification of chromosomal

environment could be involved in the complex dysregulation. Further experiments in animal models are needed to confirm these hypotheses. Although *CHN1* gene is the best candidate gene for the nystagmus, its over expression might not be the only explanation for this finding.

Conflict of interest:

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1

A: Normal hands x-rays of the affected father.

B: Hands of the daughter after surgery. Note scars after surgery for 3-4 fingers' skin syndactyly

C: Normal hands x-rays of the affected daughter.

Figure 2

Array-CGH analysis and genes involved in the 3.8 Mb wide 2q31 duplication. Comparison with the 2q31 duplication involved in the Kantaputra mesomelic dysplasia.

Figure 3

Q-PCR analysis. Estimated copy variatons for the different analysed exons of *CHN1, HOXD13, HOXD12, HOXD10* and *CHRNA1.* The confidence interval is 95% with n=4. There are 3 copies in the father and his daughter, whereas there are 2 in the mother and the control.

Figure 4

Karyotype and FISH analyses. Note tandem 2q duplication.

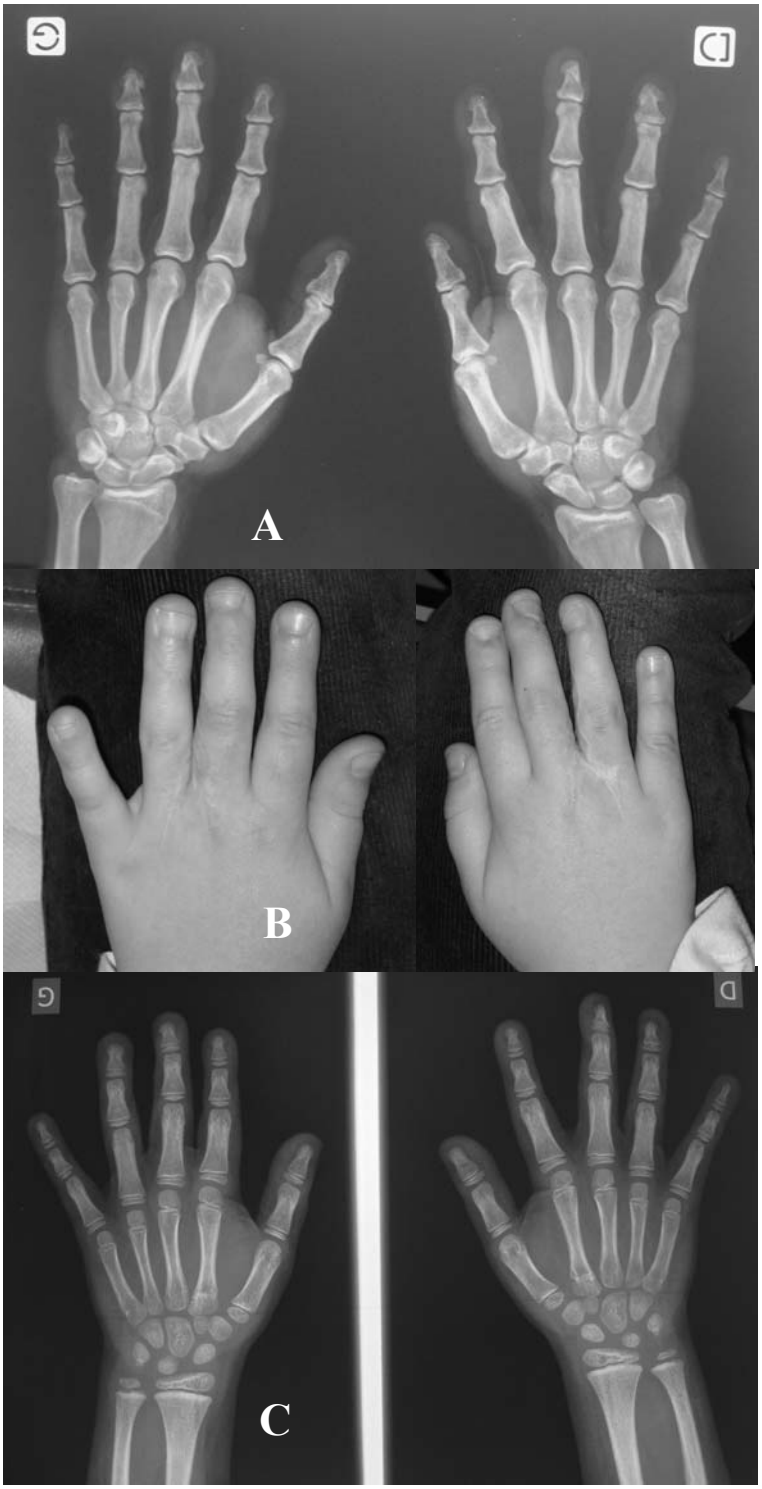
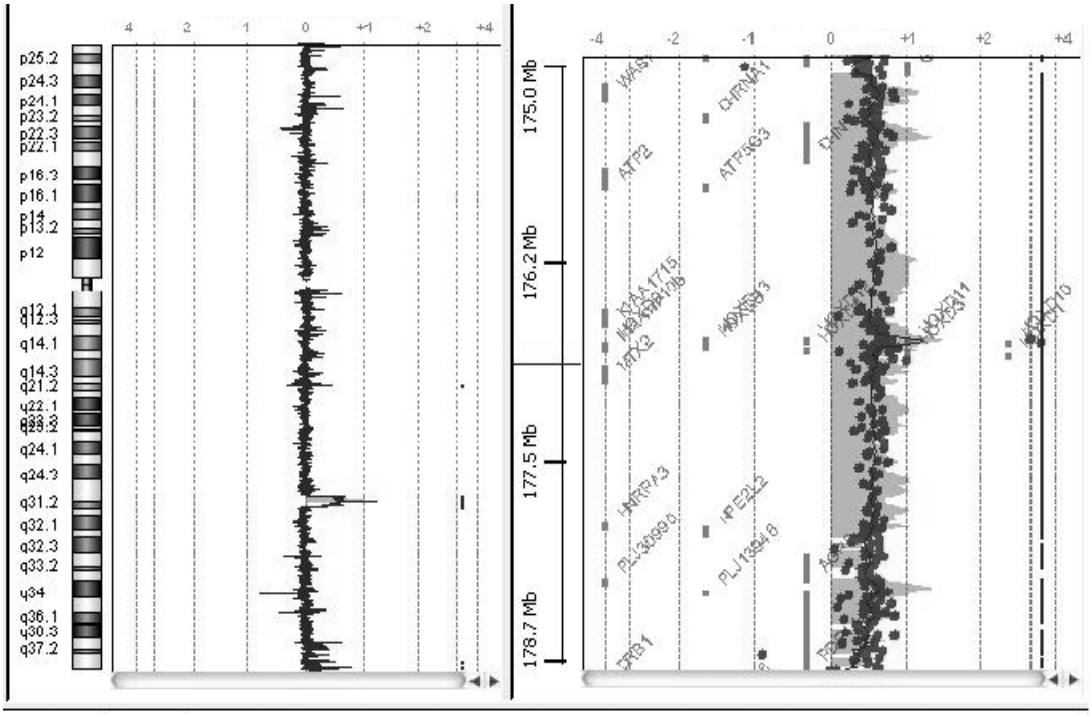


Figure 1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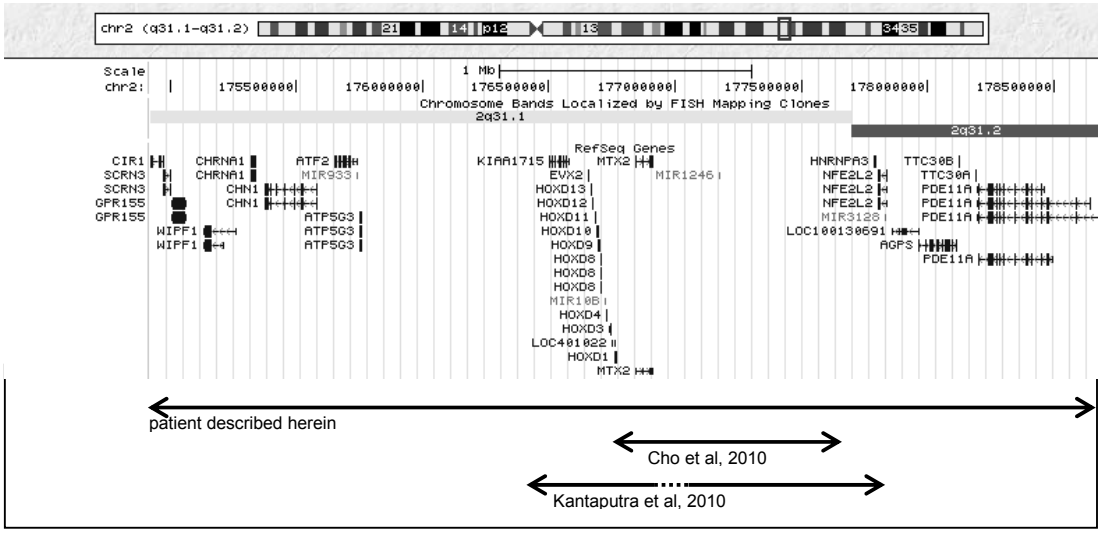
chr2 (q31.1-q31.2)
Scale
chr2:
1 Mb
175500000
176000000
176500000
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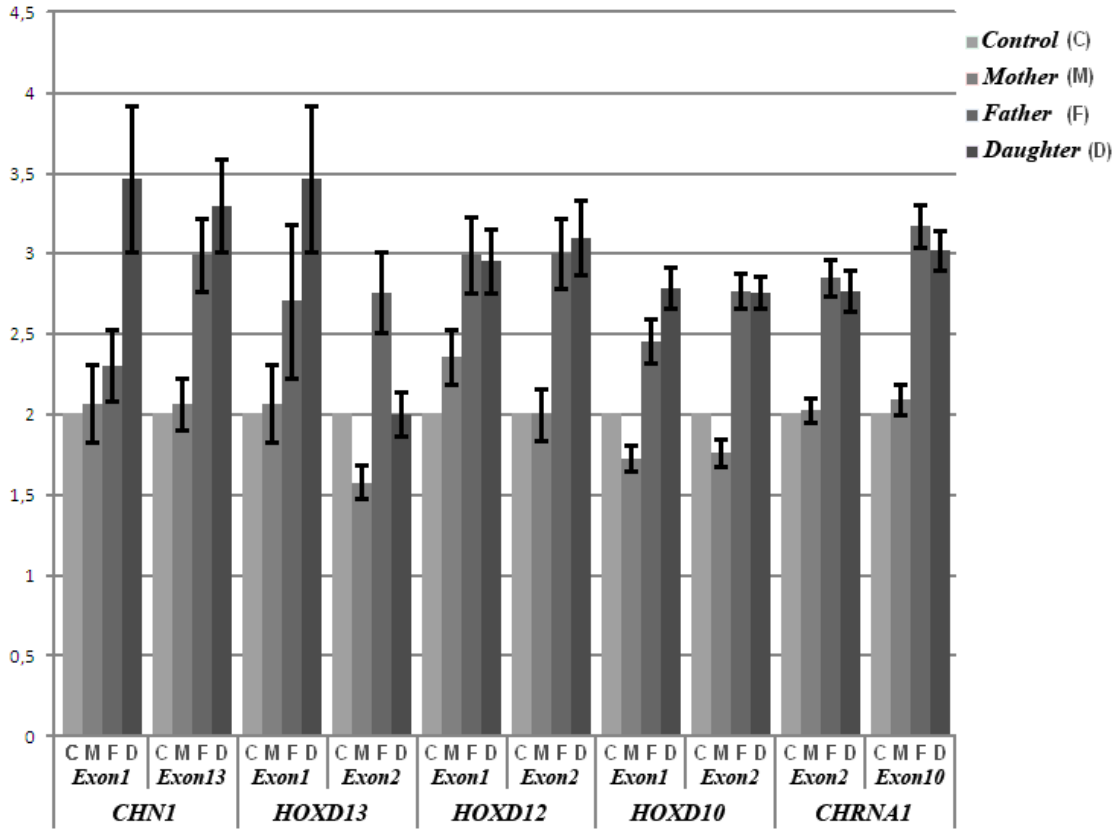
Chromosome Bands Localized by FISH Mapping Clones
2q31.1
2q31.2
RefSeq Genes
patient described herein
Cho et al, 2010
Kantaputra et al, 2010

Figure 3


4,5
4
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Control (C)
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Daughter (D)
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Exon2
Exon10
CHN1
HOXD13
HOXD12
HOXD10
CHRNA1

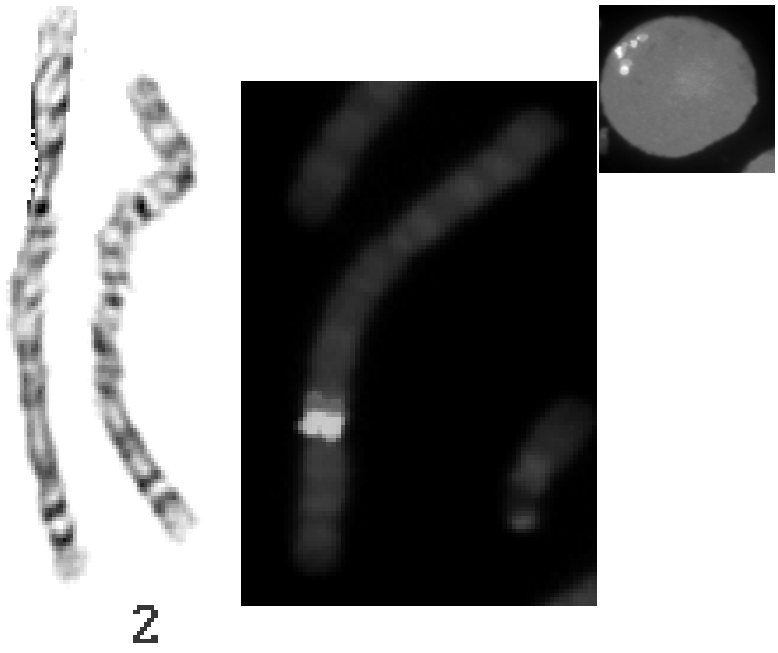

Figure 4